

Isolation of single cells from cryoinjured hearts

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An abbreviated version of this protocol was published in eLIFE in Dec 2019

Single-cell analysis uncovers that metabolic reprogramming by ErbB2 signaling is essential for cardiomyocyte proliferation in the regenerating heart

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Detailed protocol

* Keep everything at room temperature until FACS sorting, afterwards cells can be put on ice.

Protocol:

1. Prepare HBSS + Heparin mix (Ca²⁺/Mg²⁺ free, Sigma #H9394).
2. Isolate ventricles and dissect hearts in HBSS + Heparin mix. Hearts should still beat, try to remove all excess tissue such as pericardial sac, fat and blood.
3. Collect hearts in 2ml eppendorf tube (ideally 5 hearts, but ok with 7) and rinse in 1x dPBS
4. Digest hearts with 1ml collagenase 1mg/ml, resuspended in HBSS (Ca²⁺/Mg²⁺ free), on shaker at 32 degrees, 700 rpm for 30-40 minutes. Pipette hearts up and down every 10 minutes to facilitate breakdown of the heart.
5. Spin 2000 rpm for 5 minutes at room temperature.
6. Remove supernatant (make sure you don't disturb the pellet)
7. Add 1ml TrypLE express, on shaker 32 degrees, 700 rpm, 15-30 minutes. Pipette every 5 minutes up and down until single cells are obtained.
8. Prepare 4x volume of complete media (1ml/tube). Complete media = 20% FBS in HBSS (Ca²⁺/Mg²⁺ free).
9. Inactivate enzyme by adding 1 ml of complete media and strain mixture through 70 um cell strainer into a 15ml tube. Add another 1ml complete media to eppendorf tube to wash and put through strainer, repeat until 3ml complete media is added.
10. Spin 15 ml tube for 5 minutes in a rotating angle centrifuge at 2000 rpm at room temperature.
11. Discard supernatant, wash with 1ml complete media and spin for 5 minutes 2000 rpm.
12. Resuspend pellet in 500ul complete media
13. Strain cells through 40um strainer
14. Leave at room temperature in the dark.
15. Just before sorting, add viability dye. This can be DAPI 20ug/ml, 1:200 dilution; or PI 1mg/ml (Sigma P4864), 1:500 dilution.
16. Sort cells.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Bakkers, J. (2020). Isolation of single cells from cryoinjured hearts. Bio-protocol Preprint. bio-protocol.org/prep682.
2. Honkoop, H., de Bakker, D. E., Aharonov, A., Kruse, F., Shakked, A., Nguyen, P. D., de Heus, C., Garric, L., Muraro, M. J., Shoffner, A., Tessadori, F., Peterson, J. C., Noort, W., Bertozzi, A., Weidinger, G., Posthuma, G., Grün, D., van der Laarse, W. J., Klumperman, J., Jaspers, R. T., Poss, K. D., van Oudenaarden, A., Tzahor, E. and Bakkers, J. (2019). Single-cell analysis uncovers that metabolic reprogramming by ErbB2 signaling is essential for cardiomyocyte proliferation in the regenerating heart. eLIFE. DOI: [10.7554/eLife.50163](https://doi.org/10.7554/eLife.50163)

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